

UNIQUE BIOCIDES FOR THE LEATHER INDUSTRY; ESSENTIAL OIL OF OREGANO

by

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ABSTRACT

The *Origanum* species are subshrubs or perennial herbs with several stems ascending or erect, sessile or petiolate leaves and flowers in verticillasters aggregated in dense or loose spikes which are arranged in paniculate or corymbiform inflorescence.

Oregano essential oil is produced from the oregano plant through the process of steam distillation. There are a variety of species referred to as oregano, but the genus *Origanum* (tribe Mentheae, Labiatae family) is characterized by a large morphological and chemical diversity: their antimicrobial activities are different from each other. It has already been found that the *Origanum minutiflorum* essential oil has antifungal activity during pickling and wet blue processes and its effectiveness improves with increasing concentration rates.¹

In this investigation, 1% of essential oils from three different oregano species and the oil of *Foeniculum vulgare* (fennel) were tested for their antimicrobial activity in parallel with 7-25 % phenol and 4-chloro-3 methyl as commercial bactericides commonly used in the leather industry.

The results showed that three essential oils of oregano had much stronger bactericidal activity than that of the commercial bactericide and the fennel oil. These findings suggested that these oregano essential oils could be used as bactericidal agents in leather industry.

RESUMEN

Las especies de orégano son sub-arbustos o hierbas perennes con varias hojas caulinares ascendentes o erguidas, hojas ovales y pecioladas y flores agregadas en verticilastros agregados en espigas densas o vacías que se unen en inflorescencia paniculada o corimbosa. La esencia del aceite de orégano se produce por la destilación al vapor de la planta del orégano. Hay una variedad de especies designadas como orégano, pero el

género *Origanum* (tribu Mentheae, familia de las Labiadas) está caracterizado por una gran diversidad morfológica y química: sus actividades antimicrobianas son diferentes una de otra. Se ha encontrado que la esencia del aceite *Origanum minutiflorum* essential tiene actividad antihongos durante el pickelado y los procesos de Wet Blue y su eficacia mejora con el aumento de la concentración.

En esta investigación, 1% de esencias de aceites a partir de tres diversas especies de orégano y el aceite del *Foeniculum vulgare* (hinojo) fueron probados por su actividad antimicrobiana en paralelo con 7-25% de fenol y 4-cloro-3-metil como bactericidas comerciales usados comúnmente en la industria de cuero. Los resultados demostraron que las tres esencias de aceites de orégano tenían actividad bactericida mucho más fuerte que el del bactericida comercial y de la esencia de hinojo. Estos resultados sugirieron que estas esencias de aceites del orégano se podrían utilizar como agentes bactericidas en la industria de cuero.

INTRODUCTION

Microbial activities are basically responsible for further degradation of organic substances in nature leading to some undesirable consequences. To avoid such activities taking place in some industrially valued organic products, such as leather, specific care must be exercised. Under microbial attack, leather changes its characteristics and this in turn causes tremendous losses in its economic value.²

Soaking is the first process which rehydrates and cleans hides. In fact, for a short soaking period bacteria don't cause important defects but when the hides are too dry, they need long soaking periods. Longer soaking periods result in great microbial growth and sometimes this may also cause permanent damages. Various researchers have reported that a wide variety of bacteria can be isolated from the soaking water, including species of *Bacillus*, *Chromobacter*, *Pseudomonas*, *Clostridium*, *Lactobacillus* and *Serratia marcescens*.³

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Since the raw materials of the tanners are not sterile,⁴ at the start of the soaking process strict controls for microbial activities should be applied. During the soaking process, bacterial attacks to the hides lead to putrefactive odors and hair slips and depending on the extent of the damage, tiny abrasions known as 'pin pricks' may develop. The leather at this stage peels off its grains,³ and with further damage on epidermis layer, matt and lusterless grain, loss of the skin substance, etc. can also occur. For longer soaking process it is therefore necessary to use bactericidal agents to prevent such undesirable

results. However, the bactericidal agents that are currently used in the industry are generally harmful to human health and nature, and their use have been either restricted or banned in certain countries. For example, Pentachlorophenol (PCP) has been banned lately due to its toxicity problems despite the fact that it was in the past known as a common chemical widely used in control of bacterial and fungal contamination in leather industry.⁵ The use of polyhalogenated phenolic compounds (TCP / TBP) has also been restricted in certain countries to that end.⁶

TABLE I
Composition of the Essential Oils Used in the Study

| Constituent | Weight percentage (%) | | | |
|------------------------|-------------------------------------|--------------------------------------|---------------------------------------|--|
| | <i>Origanum onites</i> ⁵ | <i>Origanum sp.(1)</i> ¹⁶ | <i>Origanum sp. (2)</i> ¹⁷ | <i>Foeniculum vulgare</i> ⁸ |
| Carvacrol | 84,48 | 78,84 | 24,45 | -- |
| Gamma-terpinen | 2,58 | 1,36 | 8,57 | -- |
| Para-cymene | 2,01 | 2,03 | 2,68 | -- |
| Thymol | 1,86 | 8,9 | 12,7 | -- |
| Linalool | 1,64 | 0,35 | 0,88 | -- |
| Caryophyllene oxide | 1,52 | -- | -- | -- |
| β-bisabolene | 1,52 | 2,47 | 1,16 | -- |
| Caryophyllene | 1,07 | 1,31 | 3,69 | -- |
| (+) - Borneol | 0,79 | 0,75 | 0,39 | -- |
| Terpinen-4-ol | 0,77 | 1,09 | 18,6 | -- |
| α-terpinen | 0,67 | -- | 4,63 | -- |
| Myrcen | 0,64 | -- | 0,67 | -- |
| α-thujen | 0,46 | -- | 0,58 | -- |
| α-pinen | 0,35 | 0,28 | 0,33 | -- |
| β-phellandrene | 0,35 | 0,73 | -- | -- |
| Linalylester | 0,21 | -- | 2,8 | -- |
| Terpinolene | -- | 0,4 | 1,61 | -- |
| Terpineol | -- | -- | 4,49 | -- |
| Sabinene | -- | -- | 3,23 | -- |
| Carvacrol methyl ether | -- | -- | 1,96 | -- |
| Cis sabinene hydrate | -- | -- | 1,09 | -- |
| α-phellandrene | -- | -- | 1,03 | -- |
| Spathulenol | -- | -- | 0,77 | -- |
| Terpinen-1-ol | -- | -- | 0,64 | -- |
| Trans piperitol | -- | -- | 0,53 | -- |
| Thymyl methyl ether | -- | -- | 0,5 | -- |
| Trans sabinen hydrate | -- | -- | 0,44 | -- |
| (+)-Aromadendren | 0,21 | -- | 0,35 | -- |
| Alpha-humulen | -- | -- | 0,31 | -- |
| (E)-Anetol | -- | -- | -- | 73,7 |
| Limonene | -- | -- | -- | 9,5 |
| Carvon | -- | -- | -- | 6,5 |
| Methyl cavicol | -- | -- | -- | 3,2 |
| Anisaldehyde | -- | -- | -- | 1,7 |
| undefined | 0 | 1,48 | 0,91 | -- |

DMDTC (Dimethyldithiocarbamate), TCMTB (2 Thiocyanato methyl benzthiazole), N-OITZ (N-octyl-isothiazolinone), OPP (o-phenylphenol), PCMC (p-chloro-m-cresol), Carbendazim, Mercaptobenzothiazole, Methylenbisthiocyanate, TCP (tri-chlorophenol), p-Nitrophenol are some of the well known biocides currently used in leather industry. Among these, TCMTB is known as a skin irritant and is strongly toxic. Mercaptobenzothiazol is treated as a suspicious cardigenic agent in USA. Methylenbisthiocyanats are known to cause high acute toxicity. As can be seen from these particular examples, it is essential to discover new chemicals that can be used safely in leather industry for undesirable affects of microorganisms. The new approach is referred to as "ecologic leather production" and hence the discovery of environmentally friendly substances has gained much importance. It has been known for many years that essential oils are 100 % natural chemicals displaying varying degrees of antimicrobial activity. These oils are volatile compounds of plant secondary metabolism, and may be used as phytoprotective agents.⁷ Ibn-i Sina was the first man obtaining the essential oils by steam distillation techniques to be used further (980-1037).⁸

The objective of this study was to determine whether the essential oils could be used as alternative bactericides in the leather

industry. Essential oils of three different oregano species plus an organic fennel oil were tested in the present research for their bactericidal activities against a commonly used antimicrobial agent. This preference was based on the earlier reports pointing out to their use in food industry and health related issues as antimicrobial agents.^{1,9,10,11,12,13,14}

EXPERIMENTAL

Materials and Methods

Raw skin

Five native brand sheep (salted-dry) raw skins were selected for the investigation. From each skin, six pieces (weighing about 100 grams) were carefully cut and used in soaking tests run in parallel in the following order; 1st row contained a known bactericidal agent (phenol, 4-chloro-3 methyl sodium), 2nd, 3rd and 4th soaking rows contained three different essential oils, 5th row contained the fennel oil and the 6th row was the control respectively. A total of thirty tests were performed for the five skin samples (5x6=30).

Essential oils and bactericidal agents

The essential oils; *Origanum onites* (Turkish oregano, potmarjoram), *Origanum sp.(1)* and *Origanum sp.(2)* and fennel oil were

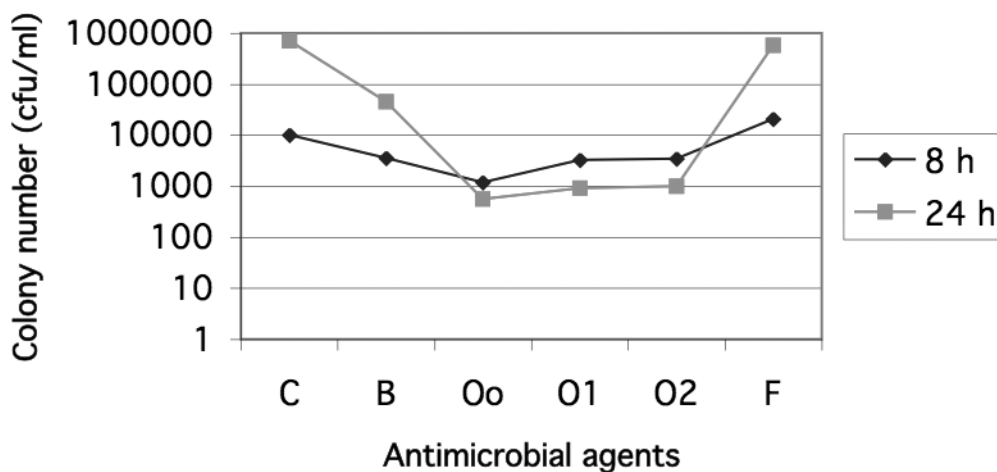


Figure 1: Comparison of the total number of bacteria by the end of 8 and 24 hours soaking periods at semi-logarithmic scale

TABLE II
Total Bacterial Counts Averaged by the End of 8 and 24 hours Soaking Periods for Six Different Skin Samples

| (cfu/ml) Product | Colony Number (cfu/ml) | |
|-----------------------------|------------------------|-------------------|
| | (8h) | (24h) |
| Control Bactericide (1%) | 3.6×10^3 | 4.6×10^4 |
| <i>Origanum onites</i> (1%) | 1.2×10^3 | 5.6×10^2 |
| <i>Origanum sp.(1)</i> (1%) | 3.3×10^3 | 9.2×10^2 |
| <i>Origanum sp.(2)</i> (1%) | 3.5×10^3 | 1.0×10^3 |
| Fennel oil (1%) | 2.1×10^4 | 5.7×10^5 |
| Control | 1×10^4 | 7.1×10^5 |

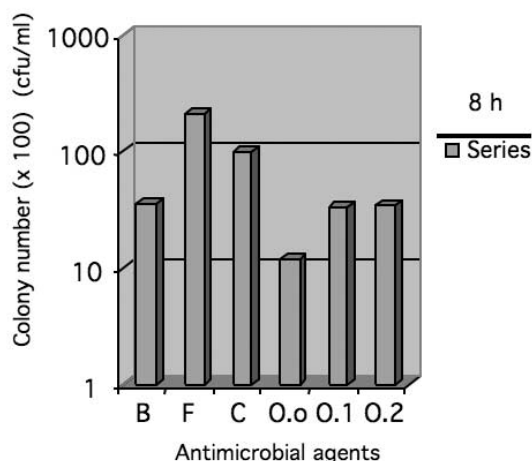


Figure-2: Total number of aerobic bacteria at the end of 8 hours soaking period

B: 1% bactericide
 F: 1% fennel oil
 C: control
 O.o.: 1% *Origanum onites* oil
 O.1: 1% *Origanum sp.* oil (1)
 O.2.: 1% *Origanum sp.* oil (2)

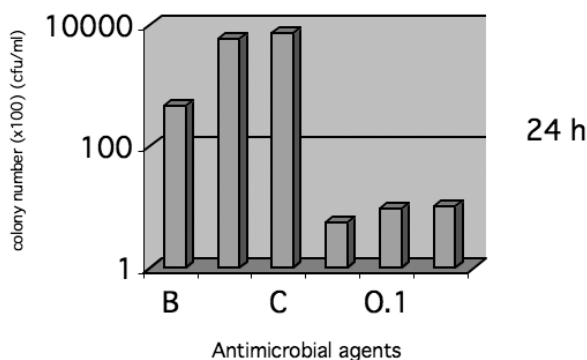


Figure 3: The total number of aerobic bacteria at the end of 24 hours soaking period

B: 1% bactericide
 F: 1% fennel oil
 C: control
 O.o.: 1% *Origanum onites* oil
 O.1: 1% *Origanum sp.* oil (1)
 O.2.: 1% *Origanum sp.* oil (2)

purchased from a commercial company. The oils were prepared in ethanol in equal volumes and the final concentration was adjusted to be 1% in soaking waters. 1% of phenol, 4-chloro-3 methyl sodium was chosen as the control bactericidal agent commonly used in leather industry.

The percentage of the constituents of the essential oils of three different oregano species and *F. vulgare* (fennel oil) is presented in Table I.^{15,16,17,18}

Soaking Process

Throughout the experiments, all washing procedures were performed in sterile distilled water (DW). Pre-washing steps (for 1h) of the leather samples and the consecutive soaking steps (8 to 24h) took place in 1L volumes of sterile DW. The aim of the pre-washing process was to let the water diffuse through the hides, removing the substances like dirt, blood, conservation salt, dust etc. Necessary additions (essential oils and the tester bactericide) were made prior to secondary soaking steps. The samples were mechanically agitated for 10 min. with 1 h intervals.

Growth Media

PCA (Plate Count Agar-Oxoid) medium was used for total bacterial counts (aerobic mesophyllics). 1 ml aliquots were taken from each row of the 5 series of soaking waters after 8 h. and 24h respectively. The aliquots were further diluted (10^1 to 10^6) in sterile saline and 0.1ml samples in duplicates were poured into PCA medium (kept ready in liquid form at 55°C in a water bath). The Petri plates were incubated for 48 hrs at 37°C and the bacterial counts were averaged and expressed as cfu/ml.

RESULTS AND DISCUSSION

Bacterial counts obtained from six different soaking tests of the five sets with 8 and 24 h intervals have been averaged and are presented in Table III. As can be seen, *Origanum onites*, *Origanum sp.*(1) and *Origanum sp.*(2) displayed varying degrees of antibacterial activity at concentrations around 1% as compared to that of the control.

The data, when examined statistically at the logarithmic scale (Fig.5), indicated that the dimension or direction of bacterial growth was not the same for all groups for 8 and 24 h soaking periods. As time proceeded, the number of bacteria increased for the bactericide, control and the fennel sample, and it decreased for *Origanum onites*, *Origanum sp.*(1) and *Origanum sp.*(2) samples respectively. This can be attributed to a meaningful group-time interaction ($p < 0.05$).

The changes observed from 8 to 24 hours in each group were applied to Paired Samples t- test. The increase in bacterial numbers for the control, the bactericide and fennel oil and, a decrease for *Origanum sp.*(1) and *Origanum sp.*(2) groups were scored meaningful. Whereas the *Origanum onites* group scored meaningless ($p = 0.196$ and $p > 0.05$).

The differences between the groups, by the end of the 8th hour, were evaluated with Oneway Anova and the differences between the groups by the end of 24th hour were organized according to the values of the 8th hour, and evaluated with Ancova (Analysis of Covariance) analysis respectively. In conclusion, the difference among the groups from the 8th to 24th hour has been found meaningful.

Examining the variations between the groups at the 8th hour, a meaningful difference was observed reciprocally between the

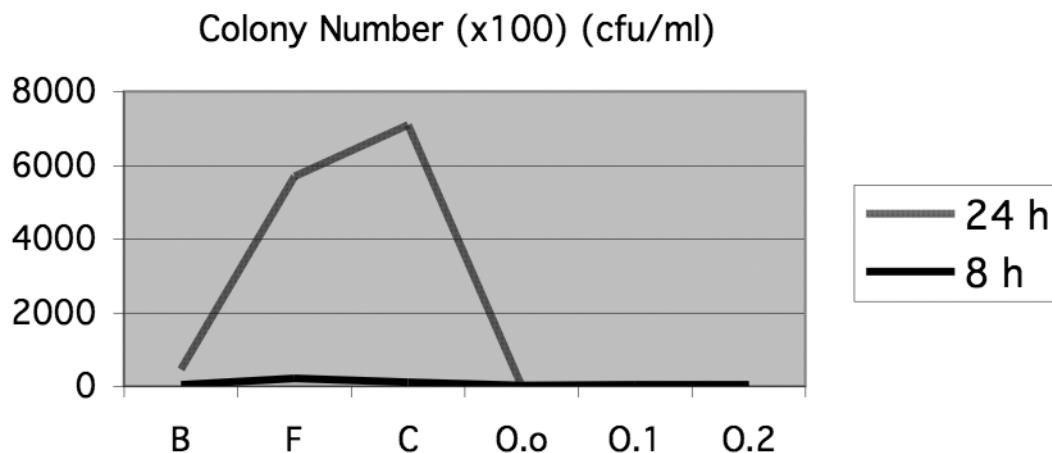


Figure 4: Comparison of the total number of bacteria between by the end of 8 to 24 h soaking periods

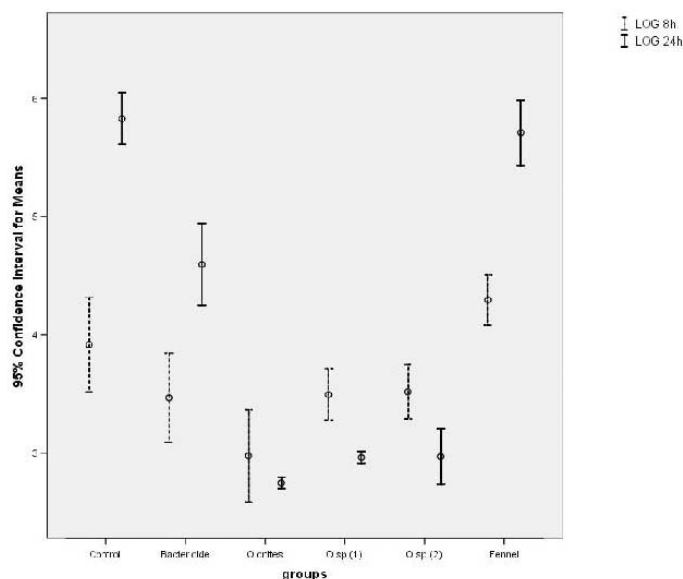


Figure 5: Comparison of the total number of bacteria by the end of 8 and 24 hours soaking periods at logarithmic scale

following; the control and *Origanum onites*, the bactericide and fennel oil, *Origanum onites* and the control, *Origanum sp.* (2)-fennel oil, *Origanum sp.*(1)-fennel oil, *Origanum sp.* (2)-*Origanum onites* and fennel oil, and finally between fennel oil and all other groups ($p < 0.05$).

The differences among the groups at the 24th hour were as follow: a meaningful difference was found among all the groups except that of the control and fennel oil ($p < 0.05$).

The differences scored as meaningful among those groups were between the following; the bactericide and all other groups, *Origanum onites* and the control, the bactericide and fennel oil, *Origanum sp.* (1) and the control, the bactericide and fennel oil, *Origanum sp.* (2) and the control, the bactericide and fennel oil.

The use of fennel oil as an antimicrobial agent has been reported¹⁴. Our results were also supported by this report. Although fennel

oil seemed to increase the bacterial growth during 8 hr soaking period, at the end of 24 hr period the bacterial counts were lesser than the control groups. The study indicated that all oregano oils used during the experiments had a greater effect than that of the bactericide and the fennel oil. The most effective oil was *Origanum onites*, of which carvacrol content was the highest (84.48%).

CONCLUSION

As a result of the study, it can be concluded that the oregano essential oils can be used as new types of bactericides which are not harmful to nature or human health due to their completely natural structure. Oregano essential oils display both antifungal and antibacterial activities and such properties are very important for their use in leather production.¹ The method of killing microorganisms by essential oils and aromatherapy has gained much emphasis especially in the field of medicine. The method is also expected to find a significant place in leather industry in near future.

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